REMARKS

Claims 1-7 and 19-25 are presently pending in the application. Reconsideration and allowance of all claims are respectfully requested in view of the following remarks.

First, the title is amended herein to more concisely reflect that which the Applicant regards as his invention. Second, amendments are made to the specification and claims as required to comply with Sequence Rules 37 C.F.R. §§ 1.821-1.825. Third, minor typographical errors in nucleic acid sequences appearing on pages 84, 125, 126 and 127 are corrected where "U" appears in a DNA sequence, or "T" appears in a mRNA sequence. Applicant's assert that these amendments do not introduce new matter within the meaning of 37 C.F.R. §§ 1.121.

It is respectfully submitted that Claims 1-7 and 19-25 are in condition for allowance and such action is hereby solicited.

If the Examiner believes there is any issue which could be resolved by a telephone or personal interview, the Examiner is respectfully requested to contact the undersigned agent at the telephone number listed below.

Respectfully submitted,

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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

The title is changed as follows.

[INTRODUCTION OF A] GLUCOSE-REGULATED mRNA INSTABILITY ELEMENT [VIA ALTERNATIVE EXON INCLUSION OF PKCBII mRNA IN VASCULAR SMOOTH MUSCLE CELLS]

IN THE SPECIFICATIONS:

The specification is changed as follows.

Page 37, first full paragraph:

2. **Tyrosine phosphatases** dephosphorylate tyrosine (Tyr) residues in proteins and share a conserved active-site sequence motif Cys-X5-Arg (X = any amino acid residue) [SEQ ID NO:1] and a Asp located in a surface loop. Protein tyrosine phosphatases (PTPs) are characterized by a signature sequence motif of 11 amino acid residues, (Ile/Val)-His-Cys-X-Ala-Gly-X-Gly-Arg-(Ser/Thr)-Gly [SEQ ID NO:2] that is found in most PTPs. The diversity within the PTPs arises from the variable N- or C-terminal sequences attached to the core catalytic domain.

Page 37, second full paragraph:

3. **Dual-specificity phosphatases** dephosphorylate Ser/Thr residues in addition to Tyr residues in proteins. Their signature motif, His-Cys-X-X-Gly-X-X-Arg-(Ser/Thr) [SEQ ID NO:3] is analogous to PTPs but these phosphatases display a restricted substrate specificity.

Page 59, first full paragraph:

Total RNA was isolated from control (5.5mM glucose) or glucose-treated (25mM glucose) A10 cells and 2 μg was used to synthesize first strand cDNA using an Oligo(dT) primer and Superscript II reverse transcriptase (Life Technologies Pre-amplification Kit). The upstream sense primer corresponded to the C4 kinase domain common to both PKCβI and PKCβII (5' CGTATATGCGGCCGCGTTGTGGGCCTGAAGGGG 3') [SEQ ID NO:4] and the downstream antisense primer was specific for PKCβI (5' GCATTCTAGTCGACAAGAGTTTGTCAGTGGGAG 3') [SEQ ID NO:5] (Chalfont *et al.*, 1995, pp.13326-13332.). These primers detect inclusion of the PKCβII exon in the mature mRNA as well as PKCβI mRNA. Sense and antisense primers for β-actin (#5402-3) were obtained from Clonetech. PCR was performed using ampliTaq Gold DNA polymerase from Perkin Elmer (#N808-0240) on 10% of the reverse transcriptase reaction product. Following 35 cycles of amplification in a Biometra Trioblock thermocycler (PKCβI and –βII: 95°C, 30 sec; 64 °C, 2min for 35 cycles; and for β-actin: 94 °C, 1 min; 58 °C, 1 min; and 72 °C, 3 min for 35 cycles), 25% of the PCR reaction was resolved on a 1.2% agarose gel. Bands were observed under UV light and photographed.

Page 60, first full paragraph:

For the stability reporter system, β-globin primers were designed. The sense primer was (5' GCATCTGTCCAGTGAGGAGAA 3') [SEQ ID NO:6] while the antisense primer for β-globin was (5' AACCAGCACGTTGCCCAGGAG 3') [SEQ ID NO:7]. PCR was performed using ampliTaq Gold DNA polymerase from Perkin Elmer (#N808-0240) on 10% of the reverse transcriptase reaction product. Following 25 cycles of amplification in a Biometra Trioblock thermocycler (94°C, 1 min; 58 °C, 1 min; and 72 °C, 3 min for 25 cycles), 25% of the PCR reaction was resolved on a 1.2% agarose gel. Bands were observed under UV light and photographed. The expected size of the amplified product was 320 bp.

Page 62, first full paragraph:

The 404bp PKCβII product was obtained by PCR amplification using sense primer to the upstream PKCβ common C4 domain (5' CGTATATGCGGCCGCGTTGTGGGCCTGAAGGGG 3') [SEQ ID NO:8] and anti-sense primer to βIV5 domain (5' GCATTCTAGTCGACAAGAGTTTGTCAGTGGGAG 3') [SEQ ID NO:9] such that the exon-included PKCβII mRNA was amplified. This PKCβII cDNA piece was cloned into the pCR-Blunt vector (Invitrogen) such that the sense transcripts could be generated from the upstream T7 RNA polymerase promoter. A 410 bp β-globin segment cloned into the pCR-Blunt vector was used as a non-specific competitor probe.

Page 84, fourth full paragraph:

5'TTTTAAACCAAAAGCTTTTTGGGCGAAACGCTGAAACTTCGACCGGTTTTTCACCC
GCCATCCACCAGTCCTAACACCTCCGACCAGGAAGTCATCAGGAATATTGACCAATC
AGAATTCGAAGGA[U]TTTCCTTTGTTAACTCTGAATTTTTAAAACCCGAAGTCAAGA
GCTAGTAGATCTGTAGACCTCCGTCCTTCATTTCTGTCATTCAAGCTCACAGCTATCA

TGAGAGACAAGCGAGACACCTCTCCCACTGACAAACTCTGTCGACTAGAATGCCCT
GAATTCTGCAGATATCCATCACACTGCG 3'

Page 84, fifth full paragraph:

Figure 27. PKC βII cDNA (350 bp) sequence [SEQ ID NO:10]

Page 125, second full paragraph:

UUUUAAACCA AAAGCUUUUU GGGCGAAACG CUGAAACUUC GACCGGUUUU
UCACCCGCCA UCCACCAGUC CUAACACCUC CGACCAGGAA GUCAUCAGGA
AUAUUGACCA AUCAGAA[TT]UUC GAAGGAUUUC CUUUGUUAAC UCUGAAUUUU
UAAAACCCGA AGUCAAGAGC UAGUAGAUCU GUAGACCUCC GUCCUUCAUU
UCUGUCAUUC AAGCUCACAG CUAUCAUGAG AGACAAGCGA GACACCUCCA
ACUUCGACAA AAGUUCACCA GGCAGCCUGU GGAACUGACU CCCACUGACA

Page 126, first full paragraph:

Figure 43. PKCβII mRNA sequence [SEQ ID NO:13] linearized at 175 bp with *Bgl II* and RNA secondary structure analysis.

Page 126, second full paragraph:

UUUUAAACCA AAAGCUUUUU GGGCGAAACG CUGAAACUUC GACCGGUUUU
UCACCCGCCA UCCACCAGUC CUAACACCUC CGACCAGGAA GUCAUCAGGA
AUAUUGACCA AUCAGAA[TT]UUC GAAGGAUUUC CUUUGUUAAC UCUGAAUUUU
UAAAACCCGA AGUCAAGAGC UAGUA

Page 127, first full paragraph:

Figure 44. PKCβII mRNA sequence [SEQ ID NO:14] linearized at 137 bp with *Hpa I* and RNA secondary structure analysis.

Page 127, first full paragraph:

UUUUAAACCA AAAGCUUUUU GGGCGAAACG CUGAAACUUC GACCGGUUUU
UCACCCGCCA UCCACCAGUC CUAACACCUC CGACCAGGAA GUCAUCAGGA
AUAUUGACCA AUCAGAA[TT]UUC GAAGGAUUUC CUUUGUU

IN THE CLAIMS:

The claims are amended as follows.

2. (Amended) The construct of claim 1 wherein the metabolite responsive instability element comprises the sequence

TAACTCTGAATTTTTAAAACCCGAAGTCAAGAGCTAGTA [SEQ ID NO:11].